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Comparison of four immune variables and pulmonary lesions of goats with intrapulmonary exposure and subsequent intrathoracic challenge exposure with *Pasteurella haemolytica*

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SUMMARY

A comparison of immune variables following lung sensitization with live *Pasteurella haemolytica* serotype 1 (Ph1)-impregnated agar beads was done in 2 separate trials. The Ph1 immune variables studied were blood bactericidal activity, serum bacteriolysis, total classical complement, and indirect hemagglutination antibody. Each trial had 16 male weanling goats: 6 controls and 10 principals. In trial 1, each goat was surgically catheterized through the trachea, then the material was deposited in a bronchus. The controls received only agar beads and the principals received agar beads impregnated with live Ph1. These goats were studied for 32 days, euthanatized, and necropsied. In trial 2, the controls were each transthoracically injected with agar beads into the left lung and the principals were similarly injected with agar beads impregnated with live Ph1. These goats were studied for 35 days, then challenge exposed transthoracically by injection of Ph1 in saline solution (1.2×10^7 CFU/ml) into the right lung. Four days later, they were euthanatized and necropsied. The volume of lung consolidated tissue was an excellent measure of Ph1 immunity. Principal goats generated solid protective immunity to subsequent challenge exposure because minimal or no lung consolidation was observed, whereas large volumes of lung consolidation were seen in the controls.

The principal goats in trial 1 gave a weak serum indirect hemagglutination Ph1 antibody response, which was attributed to the bronchial method of depositing the Ph1. The corresponding response of the control group remained negative. The Ph1 agar beads (1×10^6 CFU in 0.5 ml) protected the bacteria from immediate phagocytosis and lysis as indicated by the induced pneumonic deaths of 2 principals 5 days later. Also, live Ph1 were isolated on day 32 during necropsy of respiratory tracts of 3 principals. At necropsy, no Ph1 isolates were found in the controls. Bacteriolytic activity was not induced against Ph1 in either control or principal groups in this trial.

In trial 2, the indirect hemagglutination Ph1 antibody response of the controls remained unchanged throughout the study, but antibody titers of the principals increased to a geometric mean of 1:250 seven days after lung injection (1×10^5 CFU in 0.5 ml). Serum bacteriolytic titers on day 0 indicated that both principals and controls could be subgrouped to high or low subgroups on the basis of their bacteriolytic activity. The bacteriolytic activities of the controls remained unchanged during the experiment, and neither control subgroup was protected from Ph1 challenge exposure. Bacteriolytic activities of the high and low principal subgroups responded differently to Ph1 agar bead lung injection, but both principal subgroups were protected from lung challenge exposure. The low principal subgroup generated high titers of indirect hemagglutination Ph1 antibody, whereas, the high principal subgroup generated lower antibody titers. Total complement, serum bacteriolytic, and blood bactericidal profiles were similar in the principal group with high bacteriolytic activity. The immune factors that protected 2 principal subgroups did not appear to be associated with Ph1 serum bacteriolysis.

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Acute bovine respiratory tract disease may develop following the stress of transportation and marketing of feeder calves. This disease remains the most important disease to the feeder calf industry. Most efforts to decrease its economic impact on the industry have had limited success. The disease is complex and *Pasteurella haemolytica* serotype 1 (Ph1) is responsible for most of the deaths.[1] The efficiency of Ph1 vaccines have been questioned because of poor responses when used in the field.[2-5]

In a previous report,[6] we published clinical results of 2 trials that used goats as a model for inducing Ph1 agar bead lung infection. The first trial was studied to determine whether Ph1 incorporated into microagar beads could survive in the lung for several weeks, and the second trial was to assess lung protection afforded by Ph1 agar beads to a subsequent lung challenge exposure.

The objective of the study reported here was to investigate the responses of several host immune variables to Ph1 to determine how they were affected by an induced Ph1 lung infection that was previously reported.[6] The immune variables studied were blood bactericidal activity,

serum bacteriolysis, indirect hemagglutinating (IHA) Ph1 serum antibody, classical hemolytic complement (C) activity, and lung protection against Ph1.

Materials and Methods

Goats—Weanling male goats were purchased from the Texas A&M Experimental Station, San Angelo, Tex. They were randomly allotted to control and principal groups. The goats were purchased on 2 separate occasions and the trials were done at different times. The clinical, bacteriologic, IHA Ph1 antibody, and pathologic results have been described.[6]

Experimental design, lung catheterization, and lung injection of Ph1 microagar beads—*Pasteurella haemolytica*-1 was embedded in agar beads by the method of Cash et al.[7] A detailed description of the administration of agar beads was reported[6] and is only briefly discussed here.

In trial 1, the principals (n = 10) each were given 1×10^6 CFU in 0.50 ml of agar bead slurry via the trachea (day 0). The catheter void volume (2.1 ml) was flushed with physiologic saline solution to clear the 0.5 ml dose of product and deposit it in the bronchus of the lung. The controls (n = 6) each were given 0.5 ml of agar beads slurry similar to the principals. All goats were euthanatized and necropsied 32 days later. Euthanasia of the goats was performed by giving an IV overdose of general anesthetic and then by exsanguination. After exsanguination, the lungs could be immediately handled without bleeding.

In trial 2, 10 principals and 6 controls were used. The Ph1 agar beads (1×10^5 CFU/0.5 ml/principal) or agar beads (0.5 ml/control) alone were given by transthoracic needle injection into the diaphragmatic lobe of the right lung on day 0. A transthoracic Ph1 challenge in physiologic saline solution (1.18×10^7 CFU/ml) was injected into the diaphragmatic lobe of the left lung of all goats on day 35. All goats were euthanatized (as in trial 1) and necropsied 4 days later (day 39) to determine relative lesion scores.

Clinical variables—The goats were observed daily for clinical signs of induced respiratory tract disease. Rectal temperatures were taken daily for the first 10 days and periodically thereafter (Table 1). Total WBC counts, PCV, and differential leukocyte counts were determined periodically during the 2 trials and have been reported.[6]

Specimen collection—Blood samples were collected in heparin (15 IU/ml) for hematologic and blood bactericidal procedures. Blood for serum was allowed to clot, placed on ice, harvested, and stored at −85 C. Each serum sample was divided into 3 aliquots prior to freezing. These samples were used to determine bacteriolysis, classical C, and IHA Ph1 antibody activity. Nasal turbinate swab specimens were collected and stored at −85 C and later cultured for Ph1.

Serum bacteriolytic assay—A standard Ph1 bactericidal assay[8] was modified to use 1-ml volumes of serum in microcentrifuge tubes. Serum samples were tested in triplicate, and the results reported as percentage of killing of the in vitro Ph1 inoculum following a 2-hour incubation period. All serum samples for each goat were assayed at the same time. A laboratory serum pool made from several Ph1-immunized calves was aliquoted into 5-ml glass bottles and stored at −85 C and used to evaluate daily assay performance.

Table 1—Sampling days for intratracheal injection of *Pasteurella haemolytica* (trial 1) and transthoracic injection of *P haemolytica* (trial 2)

Variable	Trial 1 (actual day)	Trial 2 (actual day)
Blood bactericidal	−1, 17, 31	−3, 11, 32, 38
Serum bacteriolytic	0, 14, 31	−3, 3, 7, 11, 23, 32, 38
Serum complement (classical)	0, 3, 7, 14, 21, 32	0, 3, 7, 14, 23, 28, 35 39
Serum IHA Ph1 antibody	−1, 3, 7, 14, 21, 32	−3, 3, 7, 11, 23, 32, 38
Total WBC and differential counts	−7, 0, 3, 7, 14, 17, 31	−10, −3, 0, 3, 7, 11, 14, 23, 32, 35, 38, 39
Ph1 nasal turbinate isolates	0, 3, 7, 14, 21, 28, 32	0, 3, 7, 14, 23, 35, 36, 37, 39
Rectal temperature	−7, −3, 0-11, 12, 13, 14, 21, 28, 32	−7, −3, 0-11, 14, 23, 28, 32, 35-39
Necropsy	32	39

IHA = Indirect hemagglutination. Ph1 = *Pasteurella haemolytica* serotype 1.

The principals and controls of trial 2 were each retrospectively allotted to 2 subgroups (high and low percentage of Ph1 bacteriolytic activity: HBA and LBA, respectively) on the basis of their relative serum bacteriolytic values on day 0.

Blood bactericidal assay—A Ph1 bactericidal assay was used as previously described.[8] Each blood sample was tested in triplicate on the day of collection.

Serum complement assay—A standard classical hemolytic C assay[9] was conducted on each serum sample collected from principals and controls, and reported in mean CH_{50} U/ml of serum. All samples taken on various days from each goat were assayed on the same day. A laboratory serum control, made from a fresh pool of bovine serum (stored at −85 C) was included with each assay to determine daily test variation.

Serum IHA Ph1 assay—Serum titers for Ph1 were determined by IHA[1] and reported in geometric mean titers for the respective groups. All serum samples from each goat were assayed on the same day with a laboratory serum control.

Necropsy—The goats in both trials were necropsied[a] to determine the extent of lung lesions induced by the agar beads or Ph1 agar beads. In trial 2, the lung immunity was evaluated by the extent of lung lesions (consolidation in cm^3) following PH1 challenge exposure.[6,10]

Statistical analysis—Means, SEM, and *P* values were determined by use of an analysis of variance or general

[a] Necropsies and histopathology were performed by Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, Tex.

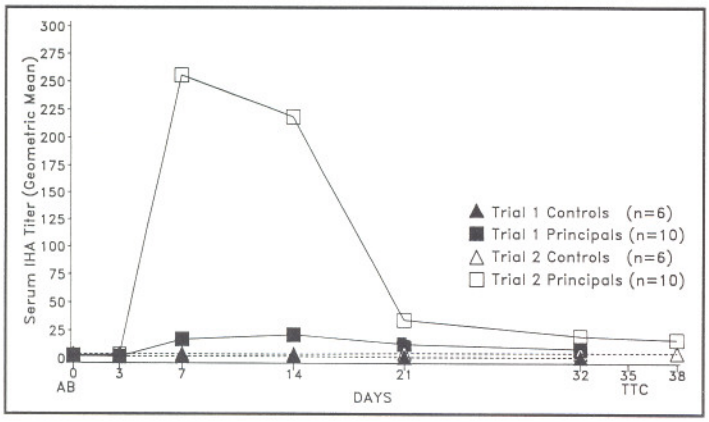

Figure 1—*Pasteurella haemolytica* (Ph1) indirect hemagglutination antibody (IHA) geometric mean titers in trials 1 and 2, following deposition of Ph1 agar beads into the lungs of principals and agar beads alone into the lungs of controls. AB = agar beads; TTC = transthoracic Ph1 challenge given on day 35 in trial 2.

linear models procedure of the statistical analysis system.[11] Differences in mean values were compared by Duncan multiple-range test. Differences were considered statistically significant at $P \leq 0.05$.

Results

Clinical, bacteriologic, and pathologic summary[6]—For this report, it is sufficient to explain that principals in both trials developed fevers (significantly above controls) for 7 days in trial 1 (peaked 40.2 C, day 3) and for 5 days in trial 2 (peaked 40.4 C, day 2) after their initial Ph1 exposure. In trial 1, the controls never became febrile, and in trial 2, the controls only became febrile after the Ph1 lung challenge. Total WBC counts revealed only small changes over the duration of both trials.

In trial 1, 2 principals died of pneumonia 5 days after Ph1 agar bead exposure. Isolants of Ph1 were recovered from their lung tissues and from the respiratory tracts of 3 principals at necropsy (day 32), but none were recovered from the controls on day 32. In trial 2, Ph1 isolants were recovered from 3 principals, but none from the controls prior to challenge exposure. After challenge exposure, the source of Ph1 could not be determined. The control group of trial 2 developed 54.9 times more consolidated lung lesions (mean, cm^3) than the principal group after the Ph1 challenge on day 35.

Serum IHA Ph1 antibody titers, trials 1 and 2—Antibody titers for all goats were low, initially, in both trials but principal titers, after Ph1 agar bead injection, rose higher in trial 2 than in trial 1 (Fig 1). In trial 2, the IHA Ph1 antibody titer of the principal subgroups reacted differently from 7 to 14 days of the experiment. The antibody titer of the LBA subgroup was tenfold higher than the HBA subgroup on day 7. The antibody titers of both principal subgroups decreased by the time of challenge on day 35. The Ph1 antibody titer of the control subgroups remained barely detectable.

Serum bacteriolytic activity against Ph1—In trial 1, there was no mean Ph1 bacteriolytic killing by either control or principal sera over the 32-day experiment (Table 2). Four sera killed Ph1 on day 0 and 2 killed Ph1 on day 14. The laboratory control serum value was within our normal laboratory range, which indicated a valid assay.

In trial 2 (Table 2), there were significant differences ($P \leq 0.003$) in bacteriolytic activity among days for principals, but not for controls. There were significant differences in bacteriolytic activity (Table 2) between high and low subgroups (both controls and principals) on sampling days 3 ($P \leq 0.04$), 7 ($P \leq 0.03$), and 14 ($P \leq 0.04$). Significance differences ($P \leq 0.05$) in bacteriolytic activity occurred among days for both principal subgroups, but not for control subgroups.

The principals and controls within each subgroup were similar on day 0 (Fig 2). The mean bacteriolytic activity among days of the HBA control group was 98%, and the mean of the LBA control group was −164% (negative values represent growth of Ph1 over the standard in vitro inoculum). The principal subgroups reacted differently in bacteriolytic activity following the induced Ph1 agar bead

Table 2—Serum bacteriolytic activity (mean % Ph1 killing ± SEM) of control and principal goats in trials 1 and 2 and of subgroups* in trial 2

Trial	Goat groups	Days 0	3	7	14	21	32	38	$\bar{x}$	*P* value
1	Controls (n = 6)	−371.0 ±86.3	NA	NA	−338.9 ±79.5	NA	−335.3 ±62.5	NA	−348.4	0.95
	Principals (n = 10)	−332.7 ±59.5	NA	NA	−388.0 ±86.1	NA	−500.1 ±60.3	NA	−406.9	0.36
	P value	0.75	NA	NA	0.70	NA	0.18	NA		
2	Controls (n = 6)	−64.2 ±51.1	−92.4 ±56.3	−24.2 ±38.4	−25.1 ±39.8	−21.6 ±37.3	−6.7 ±25.3	−14.2 ±34.6	−35.5	0.99
	Principals (n = 10)	−394.6 ±97.9	−29.2 ±24.0	66.0 ±6.6	58.5 ±7.2	13.4 ±12.3	−9.1 ±13.8	7.4 ±16.1	−41.1	0.003
	P value	0.24	0.56	0.18	0.23	0.61	0.96	0.75		
2	Subgroups*									
	High bacteriolytic control (n = 3)	97.7 ±0.9	97.3[a] ±0.6	97.1[a] ±0.7	98.8[a] ±0.2	98.8 ±0.28	98.9[a] ±0.4	96.2 ±1.5	97.8	0.73
	Low bacteriolytic control (n = 3)	−226.2 ±80.7	−282.2[b] ±82.8	−145.5[b] ±60.7	−149.0[b] ±63.8	−142.2 ±57.6	−112.4[b] ±19.9	−87.8 ±47.9	−163.6	0.93
	High bacteriolytic principal (n = 3)	89.3 ±4.0	94.6[a] ±2.0	84.0[a] ±4.7	66.4[a] ±11.1	10.1 ±18.7	21.7[a] ±13.5	55.7 ±0.9	60.3	0.05
	Low bacteriolytic principal (n = 7)	−602.0 ±120.6	−82.3[a,b] ±29.0	57.1[a] ±9.3	54.5[a] ±9.9	15.2 ±17.2	−24.6[a,b] ±19.7	−16.7 ±23.1	−85.5	0.001
	P value	0.12	0.04	0.03	0.04	0.10	0.03	0.28		

* Subgroups were based on serum bacteriolytic values on day 0 of trial 2.
Means with different superscripts are significantly different among treatment subgroups. NA = Not applicable.

Table 3—Blood bactericidal activity (mean % killing ± SEM) compared between control and principal goats in trials 1 and 2 and between subgroups* in trial 2

Trial	Goat groups	Days 0	14	32	38	$\bar{x}$	*P* value
1	Controls (n = 6)	88.5 ±1.4	98.9 ±0.3	97.4 ±0.2	NA	94.5	0.004
	Principals (n = 10)	77.9 ±4.7	91.5 ±1.1	66.8 ±3.2	NA	78.6	0.12
	P value	0.40	0.02	0.002	NA		
2	Controls (n = 6)	95.9 ±1.2	94.1 ±1.3	95.2 ±1.2	96.5 ±1.0	95.4	0.90
	Principals (n = 10)	93.4 ±0.8	93.4 ±1.2	87.8 ±1.5	95.9 ±0.7	92.6	0.08
	P value	0.37	0.84	0.10	0.79		
2	Subgroups*						
	High bacteriolytic control (n = 3)	99.9 ±0.03	99.8 ±0.01	99.4 ±0.30	99.1 ±0.28	99.5	0.53
	Low bacteriolytic control (n = 3)	91.9 ±1.9	88.4 ±0.6	91.1 ±1.6	93.9 ±1.8	91.3	0.68
	High bacteriolytic principal (n = 3)	98.3 ±0.4	95.9 ±0.9	83.9 ±3.2	96.7 ±0.7	93.7	0.06
	Low bacteriolytic principal (n = 7)	91.2 ±0.8	92.1 ±1.7	89.8 ±1.7	95.4 ±1.0	92.1	0.51
	P value	0.02	0.15	0.16	0.53		

* Subgroups were based on serum bacteriolytic values on day 0 of trial 2.
NA = Not applicable.

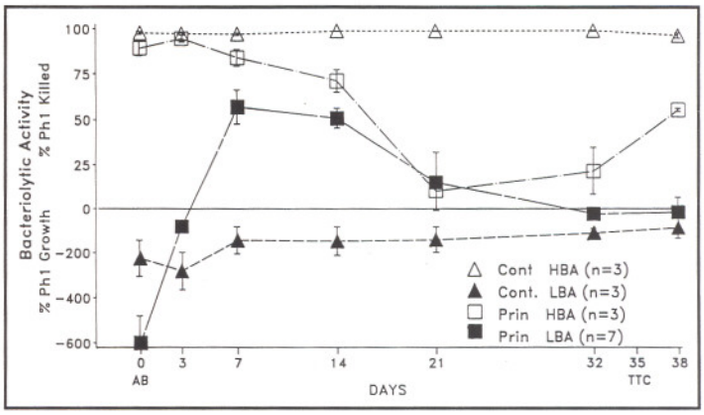

Figure 2—Serum bacteriolysis of principal and control subgroups (trial 2) on the basis of their Ph1 killing activity prior to Ph1 lung exposure. HBA = high bacteriolytic activity; LBA = low bacteriolytic activity. *See* Figure 1 for key.

lung infection. The HBA principal group decreased in activity from day 3 to day 21, then increased from day 21 to day 38. The LBA principal group increased in activity from day 0, to a maximum on day 7, then decreased through day 21, to negative values on days 32 and 38. The LBA principal subgroup activity was significantly ($P \leq 0.04$) higher than the LBA control subgroup on days 7 and 14, and tended to be higher on other posttreatment days. The bacteriolytic activity of the HBA principals tended to decrease below HBA control values after day 0.

Blood bactericidal activity against Ph1—In trial 1, blood bactericidal activity was higher ($P \leq 0.02$) in the control group than the principal group on days 14 and 32 (Table 3). In trial 2, the bactericidal activity of the high subgroups (Table 3) was higher than the low subgroups, with one exception (HBA day 32). The mean blood bactericidal activity of the HBA principal subgroup decreased to 83.9% ($P \leq 0.06$) on day 32 and recovered on day 38, 3 days after Ph1 challenge.

Classical complement activity—There were no significant differences in the C activity between controls and principals on any of the sampling days in either trial (Table 4). There were significant differences among days for the principals in both trials (Table 4). Significant differences in C activity were observed between bacteriolytic subgroups on days 21 ($P \leq 0.03$), 32 ($P \leq 0.0002$), and 35 ($P \leq 0.02$; Table 4). A significant difference ($P \leq 0.003$) was seen in mean C activity among days for the HBA principal subgroup. This subgroup decreased more in C activity on days 21, 32, and 35, compared with the other subgroups. Both HBA subgroups had lower C values than the corresponding LBA subgroups.

Comparison of variables—There was a positive relationship between C and bacteriolytic activity in the HBA principal subgroup (Fig 3). Both variables decreased from days 7 to 32, and then increased on day 38. In this subgroup, C and bacteriolytic activity had an inverse re-

Table 4—Serum complement (CH_{50} U/ml ± SEM) of control and principal goats in trials 1 and 2 and of subgroups* in trial 2

Trial	Goat groups	Days 0	3	7	14	21	32	35	38	$\bar{x}$	*P* value
1	Controls (n = 6)	69.5 ±3.3	89.0 ±3.7	85.7 ±4.0	73.3 ±5.8	79.7 ±4.2	81.3 ±3.8	NA	NA	79.9	0.53
	Principals (n = 10)	63.6 ±2.1	78.2 ±3.4	93.0 ±2.1	80.4 ±3.5	78.2 ±2.7	81.8 ±1.9	NA	NA	78.0	0.02
	P value	0.44	0.32	0.41	0.59	0.88	0.95	NA	NA		
2	Controls	92.0 ±3.9	89.0 ±1.9	91.8 ±1.9	92.4 ±1.4	90.1 ±3.1	73.1 ±2.9	84.0 ±3.2	91.1 ±5.6	87.9	0.42
	Principals	89.3 ±1.4	90.0 ±3.0	98.1 ±2.4	95.4 ±3.4	78.9 ±2.7	78.1 ±2.9	80.4 ±3.0	95.6 ±1.1	88.4	0.03
	P value	0.70	0.91	0.36	0.74	0.19	0.56	0.70	0.64		
2	Subgroups*										
	High bacteriolytic control (n = 3)	83.2 ±6.7	84.5 ±2.2	84.5 ±0.2	90.6 ±1.1	85.4[a] ±5.7	60.3[b] ±0.4	74.6[a,b] ±4.5	86.0 ±7.3	81.2	0.38
	Low bacteriolytic control (n = 3)	100.9 ±3.6	93.4 ±2.9	99.0 ±2.1	94.2 ±2.8	94.8[a] ±2.9	85.8[a] ±1.2	93.4[a] ±3.0	96.1 ±9.9	94.7	0.96
	High bacteriolytic principal (n = 3)	83.0 ±1.9	94.9 ±2.0	96.2 ±2.6	80.9 ±7.3	63.0[b] ±3.6	58.1[b] ±1.1	61.7[b] ±1.3	91.8 ±1.6	78.7	0.003
	Low bacteriolytic principal (n = 7)	91.0 ±1.6	87.8 ±4.2	99.1 ±3.5	102.6 ±2.9	88.5[a] ±0.9	88.1[a] ±2.1	89.7[a] ±2.8	97.4 ±1.4	93.0	0.39
	P value	0.36	0.85	0.43	0.28	0.03	0.0002	0.024	0.85		

* Subgroups were based on serum bacteriolytic values on day 0 of trial 2.
Means with different superscripts are significantly different among treatment subgroups. NA = Not applicable.

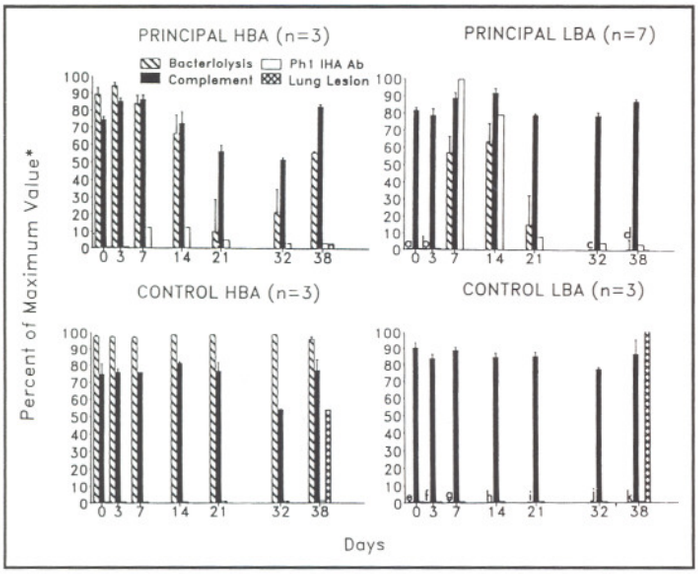

Figure 3—Serum bacteriolysis, complement, *Pasteurella* antibody, and lung lesions of subgroups in trial 2. *Pasteurella*-consolidated lung lesions were measured on day 39, but are shown with data on day 38. * Percentage of maximal values are means calculated by determining each sample as a percentage of highest value among all samples for each respective variable. a to k = negative values for LBA principal and control subgroups. *See* Figures 1 and 2 for key.

lationship to IHA Ph1 antibody, which increased through day 14, and decreased through day 38. Complement activity remained high throughout the trial for the LBA principal subgroup. Bacteriolytic activity and IHA Ph1 antibody increased through day 14, and decreased through day 38. In the control subgroups, the 3 serum variables remained unchanged.

The maximal differences in IHA Ph1 antibody for the principal subgroups did not correspond to differences in lung lesions, because both subgroups were protected. Lesions were severe in both control subgroups and neither generated IHA Ph1 antibody titers (Fig 3).

Blood bactericidal and serum bacteriolytic activity in the HBA principals were similar in that they decreased through day 32, then recovered on day 38 (Fig 3). Serum bacteriolytic activity in the HBA subgroups approximated the corresponding levels of blood bactericidal activity. The amount of serum bacteriolytic activity in the LBA subgroups were low relative to the corresponding blood bactericidal activity.

Discussion

Bactericidal activity is an important body defense mechanism against invading microbes in human beings and other animals,[12-15] although apparent contradictions of this statement have been reported. MacDonald[16] reported that bronchial alveolar wash material from sham-vaccinated calves with a serum geometric mean IHA Ph1 titer of 1:27 developed higher bactericidal killing than did Ph1-vaccinated calves with an IHA titer of 1:717. The source of the Ph1 isolate used in the assay may influence the result. Clinical isolates of Ph1 and *P multocida* were resistant to serum, and isolates from cattle without clinical signs of disease varied in serum susceptibility.[17] Unlike MacDonald,[16] we found that blood bactericidal activity in this study was not influenced by sham vaccinating the controls. However, we found 2 different goat populations concerning serum bacteriolytic activity and both were susceptible to Ph1.

The main active factors involved in specific acquired in vivo bactericidal activity are host defense cells (macrophages and microphages), specific antibody (immune opsonins or bacteriotropins), C, and the microbe (specific antigen) that induces specific antibody. The important active factors for in vitro bacteriolysis are the bactericidal factors without host defense cells. In vivo, the latter mechanism never operates alone in a normal host because phagocytic cells are always present. Few studies have tested fresh low-passaged pathogenic bacterial isolates susceptibility to lysis in serum.[18]

The use of bacteriolytic assays to evaluate or predict protective immunity suspected in a host against a specific microbe is an interesting hypothesis. We previously demonstrated,[8] retrospectively, an apparent inverse relationship between the degree of market stress and bovine Ph1 blood bactericidal activity. The more market stress applied, including longer periods of morbidity, the greater the decrease in Ph1 bactericidal activity.

Nonspecific Ph1 antibodies may be induced by other gram-negative bacteria that share some similar somatic-related epitopes.[b] The antibody induced in such a situation may have poor avidity and be of little or no importance in protecting the host against Ph1 infection.[19] This phenomenon may account for the HBA control subgroup not having protection against the Ph1 lung challenge in trial 2 (Fig 3). Other possible in vivo humoral substances (antibacteriolytic enzymes: lysozyme and β lysine) in some animals may also influence the killing of the bacteria in their bodies.[18] These humoral substances may also influence the killing of certain species of bacteria used in in vitro serum bacteriolytic assays. The nonprotective, nonspecific antibodies or other humoral substances may account for some of the apparent contradictions reported in use of these assays. Also, the bacteriolysis results would be difficult to interpret if 2 subpopulations of animals exist on the basis of their genetic capability to respond to a specific antigen.

The amount of total hemolytic C (Table 4) appears to be sufficient in all goats of both trials to complete the blood bactericidal and serum bacteriolytic assays on all sample days. In trial 2 (Fig 3), the C profile of the HBA principal subgroup paralleled its serum bacteriolytic activity, suggesting that the consumption of bacteriolytic antibody was related to Ph1 multiplication. The amount of C for both control subgroups remained constant, as would be expected because they received no Ph1, but we cannot explain why the LBA principal C concentration remained constant.

The IHA Ph1 serum antibody (Fig 1) of the principal group obtained in trial 1 was tenfold less than the principals obtained in trial 2. This titer difference was the result of administering the Ph1-impregnated agar beads via the tracheal route. The systemic antibody appeared less effected by the Ph1 tracheal route to the lung (trial 1) than by transthoracic injection of Ph1 into the lung (trial 2), even though 2 goats in trial 1 died of the infec-

[b] Tsai LH, Collins MT, Hoiby N. Cross-reactions between *Pasteurella haemolytica* and 20 other bacterial species studied by crossed immunoelectrophoresis (abstr), in *Proceedings*. Annu Meet Am Soc Microbiol 1986;R15:239.

tion 5 days after inoculation. The IHA Ph1 antibody response to lung injection (trial 2) was typical of any paranatal injection of Ph1; however, the induced lung immunity was superior to the usual IM or SC Ph1 vaccination.[20,21]

There was a substantial difference in IHA Ph1 antibody response (Fig 3) between principal subgroups; however, both subgroups were protected from the Ph1 challenge. It is clear that the Ph1 agar bead-induced infection of the lung generated a protective immunity. In this trial, it appeared that Ph1 immunity was associated with an initial increase in IHA Ph1 antibody, even though there was a tenfold difference in titer magnitude between the 2 principal subgroups. This difference may suggest that a genetic mechanism(s) is involved.

The blood bactericidal activity of the goat samples in both trials had higher killing values and less variance than the corresponding bacteriolytic activity from duplicate samples. There was a significant difference in blood bactericidal activity between controls and principals in trial 1 on sampling days 14 and 32, but no significant difference was seen in trial 2. The principals in both trials had lower bactericidal values than the controls on day 32 (Table 3). This may be the result of the Ph1 infection consuming anti-Ph1 opsonin antibodies. It was previously demonstrated[8] that market-stressed, Ph1-infected feeder calves, morbid for 6 days or more, also had depressed blood bactericidal activity. In trial 2, the blood bactericidal activity of the principal subgroups appeared to increase after challenge (Table 3). This rapid increase in Ph1 bactericidal activity was probably attributable to an anamnestic opsonic antibody response to Ph1 lung challenge in previously primed principals.

Mean serum bacteriolytic activity was not detected in trial 1 (Table 2). This implies that the tracheal route of bronchial agar bead Ph1 infection was not sufficient to stimulate a systemic serum bacteriolytic response in the principals, even though the challenge exposure killed 2 principals on day 5. This is also in agreement with the small increase in IHA Ph1 antibody of the principals.

In trial 2, on the basis of day-0 values, the bacteriolytic activity of the subgroups indicated that 2 goat subpopulations existed for both control and principals (Fig 2). This is indicated by the fact that the bacteriolytic activity of HBA and LBA controls maintained their relative positions throughout the experiment. Also, the principal subgroups reacted uniquely to the initial Ph1 agar bead injection. The bacteriolytic activity of the HBA principal subgroup decreased, apparently because of increased Ph1 antigen load after initial exposure. The bacteriolytic activity of the LBA principal subgroup increased substantially, similar to a primary antibody response. During the brief 4-day-designed[10] interval between challenge exposure and necropsy, bacteriolytic activity of the HBA principal subgroup increased like its corresponding bactericidal activity. This was apparently attributable to the reexposure of Ph1 at challenge exposure (day 35) and was similar to an anamnestic antibody response (Fig 2).

There are 3 main alternative hypotheses that might explain the HBA and LBA of these 2 different goat subpopulations. First, the HBA controls and principals had a prior exposure to Ph1. This alternative hypothesis is unacceptable on the basis of the data. All of the young goats from 1 herd were born and raised on the same ranch with the same environment. There were no data indicating that the weanling goats had any prior exposure to Ph1 before the start of the trial. This was further substantiated because the HBA and LBA controls were susceptible to Ph1 challenge at the end of trial 2 and had negative IHA Ph1 titers before and throughout trial 2. This also indicated no transfer of Ph1 from the positive controls to the negative contact controls during the experiment. The Ph1 agar bead exposure of LBA and HBA principals indicated substantial changes in bacteriolytic antibody and IHA Ph1 antibody production. However, the degree of change is probably dependent on the dose and route of administration.

Second is the hypothesis that a bacterium (gram-negative) not closely related to Ph1, induced bacteriolytic antibody in the goats capable of killing Ph1 in vitro, but provided no protective antibody in vivo against a Ph1 challenge. This hypothesis is supported by the data of the HBA controls, which had high bacteriolytic activity in vitro, but were not protected from the Ph1 in vivo challenge.

Third is the hypothesis that genetically controlled mechanisms may partially explain the presence of the 2 goat subpopulations. This is supported by: the initial existence of the subgroups; the contrasting bacteriolytic profiles and maximal IHA Ph1 antibody responses of the HBA and LBA principals after initial Ph1 exposure (Fig 3); the bacteriolytic activity of the control HBA and LBA subgroups was maintained throughout the experiment, showing that initial bacteriolytic activities were not transitory; and the bacteriolytic activity of the principal HBA and LBA subgroups had a propensity to end as they began, given sufficient time between the antigenic stimulation (Fig 2). The genetic mechanism(s) may account for a different response in HBA and LBA goats to a closely related bacterium (but not Ph1) that induces high Ph1 bacteriolytic activity in HBA goats, but not in LBA goats.

The agar beads impregnated with live Ph1 injected in the lung of goats induced solid lung protection against a subsequent challenge. Serum bacteriolysis responded to Ph1 exposure; however, in this study, it was not an indicator of Ph1 immunity because the HBA control subgroup was susceptible to challenge. In trial 2, there were 2 different subpopulations that reacted differently in the production of bacteriolytic antibody and IHA Ph1 antibody. Feeder calves should be investigated in a similar way to determine whether similar subpopulations exist in herds with common breeding. The agar bead method of inoculating the lung with live Ph1 in low doses could be used to determine the virulence of various Ph1 isolates and also the immunogenicity of various subunit vaccines.[22-24]

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